Preprint
UCRL-JC-151076

Development of a Microcantilever-Based Pathogen Detector

B. L. Weeks, J. Camarero, A. Noy, A. E. Miller J. J. De Yoreo

This article was submitted to Nanotech 2003, San Francisco, CA
February 23 – 27, 2003

November 18,2002

Development of a microcantilever-based pathogen detector

B.L. Weeks, J. Camarero, A. Noy, A.E. Miller and J.J. De Yoreo

Lawrence Livermore National Laboratory, Livermore, CA 94550

ABSTRACT

The ability to detect small amounts of materials, especially whole organisms, is important for medical diagnostics and national security issues. Engineered micro-mechanical systems can serve as multifunctional, highly sensitive, real time, immunospecific biological detectors under certain circumstances.

We present qualitative detection of specific *Salmonella* strains using a functionalized silicon nitride microcantilever. Detection is achieved due to differential surface stress on the cantilever surface in-situ. Scanning electron micrographs indicate that less than 25 adsorbed bacteria are required for detection.

Keywords: Microfabrication; Cantilever; Biosensor; Immunosensor; Surface functionalization

1 INTRODUCTION

The atomic-force microscope (AFM) cantilever is not only crucially importance in scanning probe microscopes has proven to be indispensable in many surface science applications. In addition AFM micromachined cantilevers are an important component in many micromechanical sensors. Their small size results in very sensitive, real-time, measurements in fluids and/or air. Recent experiments have used AFM cantilevers as versatile sensors to distinguish between DNA oligonucleotides,[1] to measure pH changes,[2] and to measure the surface stress associated with molecular adsorption[3]or absorption.[4] In many of these applications, the deflection of the cantilever is driven by the build up of surface stress as its surface is modified. The surfaces stress associated with a cantilevers deflection can be predicted by Stoney's formula and has been investigated in detail.[5]

An alternative approach for detection is based on dynamic interrogation using resonant shift. In this approach, additional mass loading results in a change of the resonant frequency of the cantilever system. This technique has been successful in the detection of single microbes of E. coli.[6] Although resonant frequency shift yields the highest sensitivity the method has many drawbacks such as low quality factor in liquids and the position of the microbe along the cantilever affects the frequency shift. The low quality factor minimizes real-time use in liquids due to the broadening of the resonant peak and experiments thus far have focused on measuring the resonance shift in air (or vacuum) after exposure to the matrix.

Here we report a real time method for the detection of whole organisms based on the change in surface stress of the cantilever.

2 EXPERIMENTAL

2.1 Experimental Setup

Experiments were performed in a Digital Instruments Mutimode Instrument (Santa Barbara, CA). A low-power He-Ne laser (<3 mW power) is focused onto the tip of the cantilever. The laser beam reflected off the cantilever is directed into a position-sensitive diode (PSD) that can detect the vertical position of a laser beam. A fluid cell—commercially available from Digital Instruments within which the cantilever is mounted, forms a 100 mL cavity. V shaped cantilevers (TM Microscopes, Sunnyvale, CA) were used in all experiments. Results presented were collected on the A cantilever which is 180 mm long and the width is 18mm. The nomilar spring constant is 0.05 N/m. Thermal effects were neglected in these experiments, however a temperature control system is available to maintain a set temperature of ± 0.05 oC.

2.2 Reagents

Dithiobis(sulfosuccinimidylpropionate) (DTSSP), obtained from Pierce Chemical Company (Rockford, IL), is a water-soluble, homobifunctional N-hydroxysuccimide (NHS) ester. It is thiol-cleavable and widely used for conjugating radiolabeled ligands to cell surface receptors. Anti-salmonella antibody (ASA) was supplied from the United States Department of Agriculture (Albany, CA) and used as supplied. Affinity-purified BSA was ordered from Pierce Chemical Company. HSA and HP were bought from Academy Biomedical (Houston, TX). Other chemicals were purchased from Sigma- Aldrich (St. Louis, MO). Two specific strains of Salmonella were investigated (Heidelberg and Typherium). Both supplied (pathogenically dead) by USDA along with monoclonal antibodies to the specific strains.

2.3 Cantilever Functionalization

The cantilevers were supplied with a gold coating for reflectivity. To remove contaminates from the surfaces the cantilevers were cleaned for 10-15 minutes in SC-1 solution (1:1:5 – NH4OH:HOOH:H2O) at 80 oC. The cantilevers were subsequently cantilever was rinsed with deionized water for 10 min. This process was done immediately before the experiments. The fluid cell and glass slide were cleaned using standard detergent for glassware and rinsed with large amounts of deionized water for about 10 min. Functionalizing cantilever with DTSSP. DTSSP was dissolved in 5 mM sodium citrate buffer (pH = 5.0) at a concentration of 1.5 mM just before use because DTSSP is moisture-sensitive. Cantilevers immersed in this solution for about 2 h at room temperature results in strong adherence of DTSSP to the gold surface by a disulfide linkage31. After derivatizing with DTSSP, the cantilevers were rinsed with 20 mM sodium phosphate buffer, 0.15 M NaCl and pH 7.5 (PBS) for 5 min and then immersed in ASA solution for at least 5 h at room temperature. The cantilever was washed by 1 mg/ml BSA solution in PBS (BSA/PBS) thrice for about 10 min and stored in BSA–PBS solution overnight at room temperature. The BSA solution is used to 'cap' any exposed areas of the cantilever to prohibit non-specific binding.

2.4 Procedure

The functionalized cantilever was mounted onto the fluid cell and equilibrated in BSA–PBS solution until a stable baseline of cantilever deflection was obtained (usually around 1 h). The control (such as BSA–PBS or a nonspecific strain of salmonella) was then injected into the fluid cell and cantilever deflection was monitored in situ. All the experiments were carried out at room temperature. Although there is some thermal drift in the experiment this did not affect the binding or results. Because there was no flow through the fluid chamber, the reaction happened in a static environment. The deflection was monitored real-time using LabView to record the signal from the photodetector.

3 RESULTS/DISCUSSION

Figure 1 shows the cantilever a typical result from an experiment. The cantilever was functionalized with the anti *S. Heidb.* antibody. The large spike at the beginning of each run is due to the injection of Salmonella (or buffer) solution creating a hydrodynamic pulse and a subsequent deflection of the cantilever. The first injection was the *S. Typh.* strain at a concentration of 100 colony forming units (cfu)/ml. No deflection of the cantilever is detected over a period of hours. This is the same result obtained with a PBS buffer solution which was used to remove the residual *S. Typh.* A subsequent injection of *S.Heidb.* causes a deflection of the cantilever within 20 seconds.

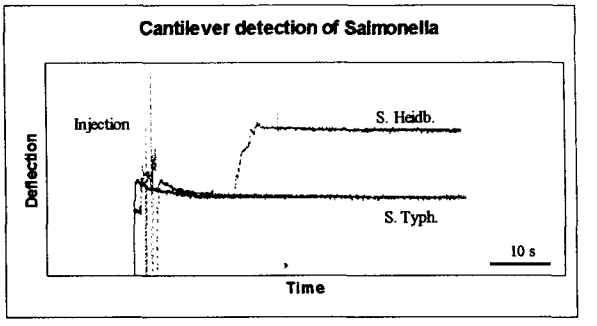

Figure 1: Typical experiment showing the detection of a specific strain of Salmonella.

Determining the detection limits of this technique is complex since we are not using a flow cell and the diffusion of Salmonella bacteria is minimal. Experiments were run by varying the concentration of the Salmonella (Figure 2).

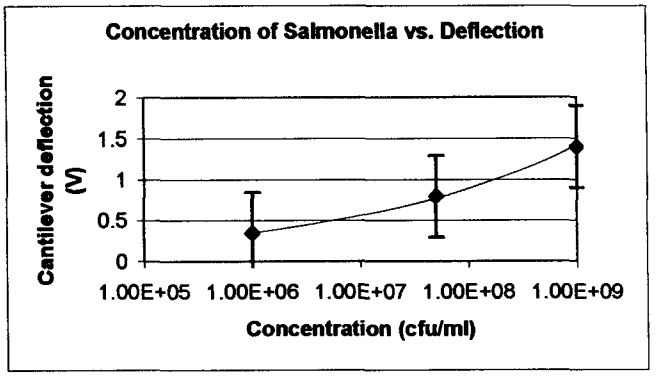

Figure 2: Dependence of concentration on cantilever deflection for *S. Heidb.*

In Addition to measuring the concentration, the size of the Salmonella bacteria yields a convenient methods of counting the number attached and relating this number back to deflection. The bacteria were counted by imaging the cantilever after experiments were complete in a field emission SEM. Images were first collected on the unfunctionalized side of the cantilever which showed no binding of bacteria. The functionalized side produced clear images of bacteria as shown in figure 3 when the complimentary strain of Salmonella was exposed to the antibody specific for that strain. Unspecific binding was not observed.

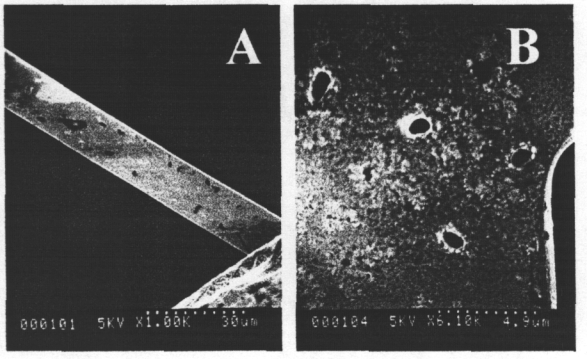

Figure 3: A: SEM micrograph clearly shows that Salmonella is attached to the cantilever surface funtionalized with the antibody. No Salmonella was observed in the unfunctionalized areas. B: Close up of *S Heidb.* on cantilever.

Figure 4 shows a graph of the number of bacteria adsorbed on the cantilever surface in relationship to the observed deflection. The cantilevers are the same as used in Figure 2. Clearly the number of bacteria plays a roll on the observed deflection of the cantilever. The large error bars are the result of a number of factors which are not controllable in our experiments such as: bacteria absorbed at the base of the cantilever contribute to the deflection more than those at the end and different cantilevers were used for each run (different spring constants). However, by fitting a trend line a few interesting points stand out. First, the smallest number of bacteria we were able to count on a cantilever, which yielded a discernable deflection, was 25 and the saturation of the cantilever deflection was about 3.5 V when exposed to high concentrations of bacteria.

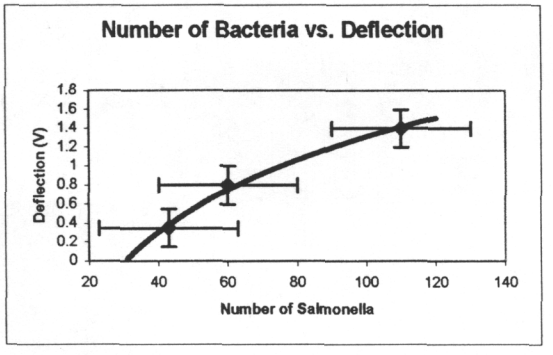

Figure 4: Graph showing the dependence of cantilever deflection on the number of bacteria counted.

4 CONCLUSIONS

We have demonstrated the use of a microcantilever for the detection of whole pathogenic organisms. The advantages of such a system are: High sensitivity and selectivity, small quantities are needed for analysis, real time detection in fluids and/or air, possibility to create portable and possible implantable devices. In addition, the fabrication process provides: batch fabrication (reduced costs), arrays of cantilevers can be fabricated for detection of multiple pathogens, and they can be integrated with other semiconductior processes (CMOS) to produce 'on chip' complete sensing devices.

5 ACKNOWLEDGEMENTS

The authors gratefully that Larry Stanker at the USDA for providing the Salmonella and anti-Salmonella antibodies for these studies. This work performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under contract number W-7405-Eng-48.

REFERENCES

[1] J. Fritz, M.K. Baller, H.P. Lang, H. Rothuizen, P. Vettiger, E. Meyer, H.J. Guntherodt, Ch. Gerber, and J.K. Gimzewski, Science 288, 316 (2000)

[2] J. Fritz, M.K. Baller, H.P. Lang, T. Strunz, E. Meyer, H.J. Guntherodt, E. Delmarche, Ch. Gerber, and J.K. Gimzewski, Langmuir 16, 9694 (2000)

[3] T. Thundat, E.A. Wachter, S.L. Sharp, and R.J. Warmack, Appl. Phys. Lett. 66, 1695 (1995)

[4] M.K. Baller, H.P. Lang, J. Fritz, Ch. Gerber, J.K. Gimzewski, U. Drechsler, H. Rothuizen, M. Despont, P. Vettiger, F.M. Battiston, J.P. Ramseyer, P. Fornaro, E. Mayer, and H.J. Guntherodt, Ultramicroscopy 82, 1 (2000)

[5] G.G. Stoney, Proc. R. Soc. London, Ser. A 82, 172 (1909)

[6] B. Ilic, D. Czaplewski, M. Zalalutdinav, H.G. Craighead, P. Neuzil, C. Campagnolo, and C. Batt, J. Vac. Sci. Technol. B 19, 2825 (2001)